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Genic determination of the autogamy trait in the hypotrich ciliate, *Euplotes crassus*

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SUMMARY

Cross-fertilizing stocks of *Euplotes crassus*, differing as to the ability to undergo autogamy, were subjected to breeding analysis. The resulting data are consistent with the interpretation that a single locus (*a*) with a pair of alleles (a^+; a^-) controls the ability to undergo autogamy. The two alleles express a simple dominance relationship in which the dominant allele (a^+) permits expression of the autogamy trait. Cross-breeding experiments also suggest that the *a* locus is not linked to the *mt* locus which determines the mating-type trait.

1. INTRODUCTION

Sexual processes in ciliates take the form of either conjugation or autogamy. While the former process involves association of two individuals during meiosis and reciprocal cross-fertilization, the latter is characterized by the production, also through meiosis, and fusion of gamete nuclei inside a single, i.e. unpaired, individual.

Naturally occurring autogamy has thus far been found in relatively few ciliate taxa (see Luporini & Dini, 1977, for detailed references). In some of these species, for example the *Paramecium aurelia* sibling-species complex (Sonneborn, 1975), autogamy occurs along with conjugation in all stocks collected, whereas in some others, for example *Euplotes woodruffi* (Kosaka, 1973), different stocks bring about only one of the two sexual processes according to the environment in which they live. In *Euplotes crassus*, a sand-dwelling species of the marine intertidal zone, several wild stocks able to reproduce both autogamically and by conjugation have been collected recently, along with sympatric stocks able to undergo only conjugation (Luporini & Dini, 1977).

The same sequence of nuclear events occurs in both sexual phenomena manifested by the marine species of *Euplotes*, except that cross-fertilization is impossible in autogamy because the cells are not united in pairs (see reviews of Raikov, 1972; Grell, 1973; Dini & Luporini, unpublished observations). The micronucleus of each cell involved in the sexual process first undergoes a preliminary mitosis,

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after the two products pass through meiosis. Of the eight derivatives of the meiotic division, six degenerate and two divide once more mitotically, giving rise to four pronuclei. Two of these degenerate while the remaining two differentiate into functional gamete nuclei which fuse to form the synkaryon. The normal nuclear apparatus is then reconstituted by the differentiation of a new macro- and micronucleus from mitotic products of the synkaryon. If the cell undergoing sexual reorganization is heterozygous (D*d*) and each degenerative process affects nuclei at random, then the probabilities of the functional gamete nuclei being *D–D*, *d–d* and *D–d* are 13/42, 13/42, 16/42, respectively. Accordingly, when autogamy takes place in a heterozygous clone, the total proportion of exautogamous homozygous recessives (*dd*) would be 13/42, the remainder being dominant (13/42 homozygous (*DD*) and 16/42 heterozygous (*Dd*)). (For the calculation of the reported ratios see Sonneborn and Beheme *in* Nobili & Luporini, 1967.) In this respect, *Euplotes* differs from *Paramecium* in which heterozygotes undergoing autogamy produce only dominant and recessive homozygotes in the 1:1 ratio. Additionally, the capacity of heterozygous cells to mature genetically different gamete nuclei enables *Euplotes* to escape the general rule of the isogenicity of the exconjugants of each pair. For instance, if one mate of a conjugating pair forms gamete nuclei with identical alleles, e.g. *d–d*, and the other forms genetically different gamete nuclei (*D–d*), after cross-fertilization one of the exconjugants will be heterozygous (*Dd*) and the other homozygous (*dd*). These notions represented the cytogenetic background utilized by Luporini & Dini (1977) to interpret the results of the breeding experiments involving autogamous and non-autogamous stocks of *E. crassus*. Such results were obtained by using a chromosomal marker gene that determined mating types and showed that genes flow between the two types of stocks. This possibility of exchanging genes permitted us to analyse the nature of the cell differences existing between the autogamous and non-autogamous stocks through a series of selected crosses.

The object of the present study was to investigate the mechanism of determination of the autogamy trait in *E. crassus*, using a breeding analysis which included cross-breeding experiments as well as a genetic analysis of the exautogamous generations after crossing. The resulting data suggest that there is a single-gene basis for the autogamy trait.

2. MATERIALS AND METHODS

Four inter-fertile stocks of *Euplotes crassus* isolated from nature were used in the breeding experiments: stocks G_{VIII}, G_{VI}, G_{12} and C_8, all used previously (Dini & Luporini, 1976; Luporini & Dini, 1977). Two were autogamous (A^+) stocks, G_{VIII} and G_{VI}; two were non-autogamous (A^-), G_{12} and C_8. These four stocks were selected for genetic analysis because of the higher viability of their F_1 offspring compared to other combinations between available A^+ and A^- stocks. They belonged to a multiple mating-type system controlled by one-locus multiple alleles which, according to the model proposed by Heckmann (1963), may be arranged in a

linear series of increasing dominance (Luporini & Dini, 1977); a heterozygote manifests the mating type of the allele with the highest rank in the series. These stocks differed in their mating types, except for the A^+ stocks which shared the same mating type. The system for designating and numbering mating types and alleles at the *mt* locus is that of Luporini & Dini (1977). The mating type expressed by each wild stock was indicated by the letter P (= Parental) followed by a number or a Greek letter, the same that labelled the respective coding *mt* allelle. Mating types coded by the *mt* alleles carried as recessive by the wild stocks were indicated by nP (= non-Parental). Since a complete dominance relationship exists between the members of the multiple-allele series, the recessive allele is not responsible for the realization of the mating type in the heterozygous state, but it is in combination with alleles occupying the same or lower rank in the series. Cells of the A^- C_8 stock were marked by their mate-killing ability, i.e. they developed properties that were lethal to 'sensitive' conjugating partners (Dini & Luporini, 1976). Mating tests supported by labelling experiments showed that the C_8 stock expressed a powerful mate-killing action against the sensitive A^+ stocks (Luporini & Dini, in preparation). From the two co-conjugants, the C_8 partner generally developed into a healthy-looking exconjugant while the autogamous one became quiescent, visibly vacuolated and rapidly died. In the cases in which a unilateral death occurred, no exception to this rule was found. Thus the C_8 stock was used to obtain homogeneous generations of progeny clones belonging to the same cytoplasmic lineage as the C_8 parent. The number of exconjugants that died because of mate-killing action is not reported in Table 1 since, at first glance, their death was not due to interstock genetic incompatibility, as was the case in the other crosses involving non-mate-killing stocks.

Stocks and progeny clones of crosses were grown in Erd-Schreiber sea-water medium inoculated with the alga *Dunaliella salina* (Heckmann, 1963). Culture procedures, methods of crossing and assessing survival of progeny after conjugation or autogamy have been described previously (Luporini & Dini, 1977; Dini & Luporini, 1979). Each progeny clone, started from one single exconjugant or autogamous cell, was kept in a 1 ml depression of a three-spot depression slide and was permitted to reproduce at its maximal fission rate. Before the algal food was exhausted, two cells of the clone were transferred *seriatim* to a new depression. After cell re-isolation, the remaining culture was divided into two depression wells with sufficient food to permit some more fissions. One of the depression-slide subcultures was used for mating-type determinations. For this purpose it was mixed with both parents, and pairs were sought in the mixtures. The progeny clone was assigned the mating type of the parent with which no reaction was observed. If a progeny clone reacted with both parents, it was assigned the nP mating type. The other depression-slide subculture was allowed to starve to induce autogamy. Under a Wild M3 stereomicroscope (35×), the autogamous cells of *E. crassus*, like early exconjugants, were easily distinguishable *in vivo* from the vegetative cells by the presence of a transparent disk (the macronuclear anlage) in the centre of the body. Thus the presence of cells with macronuclear

anlagen in the unmixed subculture was evidence that autogamy had occurred in single cells. The ability of progeny clones to undergo autogamy was tested throughout a period of three months, which is, as a rule, a time span far exceeding the immature period of healthy and normally growing (about two fissions per day) clones of *E. crassus* (Luporini & Dini, 1977). This time limit was imposed since alterations of cell viability had been expressed in clones maintained for three months at their maximal fission rate.

In most ciliates both exconjugants are genetically identical. Consequently, the co-conjugant clones derived from them are always phenotypically identical for any trait under direct genetic control (synclonal inheritance). Owing to the cytogenetic peculiarities of the conjugation process in *E. crassus* (see Introduction), however, the co-conjugant clones can be phenotypically different (clonal inheritance) (Heckmann, 1964; see also Luporini & Dini, 1977). Hence, the genetic unit to be used in presenting the data concerning both the autogamy and mating-type traits has been taken to be the exconjugant clone.

In some cases the segregations obtained were subjected to a Chi-square test for homogeneity. Such a test was performed following the method described by Brandt and Snedecor as reported in Scossiroli & Palenzona (1971). This method permits one to test the homogeneity of the segregations independently from the expected frequencies.

3. RESULTS

A programme of crosses was performed to determine if gene differences between autogamous (A^+) and non-autogamous (A^-) wild stocks accounted for the difference in their ability to undergo autogamy. The crossing scheme was the following: (*a*) A^+ stocks were crossed to A^- stocks to form F_1s; (*b*) A^+F_1 clones were backcrossed to the respective A^- parents to yield B_1s; (*c*) A^+F_1 clones of the same offspring were intercrossed to obtain F_2s. Other indications concerning the control system of autogamy derive from the genetic analysis of the exautogamous F_2s obtained by passing A^+F_1 clones through autogamy.

Since the genotypes and the order of dominance of alleles at the *mt* locus of the four selected stocks were previously known (Luporini & Dini, 1977), the *mt* gene was taken as a marker. The mating-type segregation patterns were checked in all offspring to determine whether the cross-fertilization at conjugation had occurred regularly.

(A) *Cross-breeding analysis*

Table 1 summarizes the results obtained from the whole series of crosses (*a*), (*b*), and (*c*). The viability of the offspring after conjugation can be deduced by comparing the 'total isolated' with the 'total examined' columns. The frequencies of segregation for the autogamy and the mating-type traits are reported in the respective columns. For both of these phenotypic segregations, the probability values (P) are for Chi-square tests performed on the expected ratios. The mating-type data from all crosses are classified according to the parental characteristics reported in the column labelled 'mating type and genotype'. The numbers of

Table 1. *Distribution of autogamy trait and mating types among progeny of various crosses of* Euplotes crassus

		Analysis of progeny clones														
				Autogamy*					Mating type							
	Mating type and genotype	Total isolated	Total examined	A+	A−	?	Expected† ratio	*P*	P-22	P-y	P-α	nP‡	?	Expected ratio	*P*
							(*a*) Original crosses								
$G_{VIII} \times G_{12}$	P-y × P-22 mt^{y}/mt^{y} mt^{22}/mt^{3}	110	88	84	2	2	1:0	—	41	46			1	1:1	0·7–0·5
$G_{VI} \times C_{8}$	P-y × P-α mt^{y}/mt^{1} mt^{α}/mt^{β}	63	42	39	0	3	1:0	—		23	6	11	2	2:1:1	0·5–0·3
							(*b*) Backcrosses								
32-$F_1 \times G_{12}$	P-y × P-22 mt^{y}/mt^{3} mt^{22}/mt^{3}	180	130	58	67	5	1:1	0·5–0·3	57	34		36	3	2:1:1	0·5
9-$F_1 \times C_{8}$	P-y × P-α mt^{y}/mt^{α} mt^{α}/mt^{β}	123	74	26	45	3	1:1	0·05–0·01		34	38		2	1:1	0·7–0·5
							(*c*) Intercrosses								
32-$F_1 \times$ 27-F_1	P-y × P-22 mt^{y}/mt^{3} mt^{22}/mt^{y}	84	52	32	15	5	3:1	0·3–0·2	28	20			4	1:1	0·3–0·2
32-$F_1 \times$ 28-F_1	P-y × P-22 mt^{y}/mt^{3} mt^{22}/mt^{y}	72	43	25	14	4	3:1	0·2–0·1	17	24			2	1:1	0·3–0·2

* A clone's phenotype is designed A+ or A− for ability or inability to undergo autogamy.

† The expected ratio for each generation was inferred by assuming that a single pair of alleles was controlling the ability to undergo autogamy.

‡ Non-parental mating type coded by an *mt* allele carried as 'recessive' by one or both parents involved in the cross.

? Unclassified clones.

clones reported in the 'autogamy' and 'mating type' columns under the question mark refer to unclassified clones. Clones were unclassified with respect to autogamy when they died for various technical reasons within the period of observation without expressing the autogamy trait. Also clones which did not react with any of the parents were considered as unclassified for mating-type trait. In each cross the total of clones typed for autogamy disagrees with the total of clones typed for mating-type trait. This discrepancy is due to two causes with counterbalancing effects but of different quantitative importance: (1) several A^+ clones, classified for autogamy, could not be typed for their mating type, while (2) several or all clones unclassified for autogamy were scored for their mating type.

(*a*) *Original crosses*

(i) The results obtained from the cross between $A^+ G_{VIII}$ and $A^- G_{12}$ are reported in Table 1, section (*a*). Fifty-five conjugating pairs were isolated but only 88 out of a possible total of 110 exconjugant clones survived and were examined. Eighty-six of these F_1 clones were typed; 84 were found to be A^+ and two A^-. These two exceptional A^- clones were derived from the mates of the same pair. The mating-type analysis of the exconjugant clones confirmed that heterotypic pairing followed by reciprocal nuclear exchange between mates had usually occurred. In fact, of the 88 F_1 clones examined, 41 showed the mating type P-22 of the G_{12} parent and 46 the mating type P-y of the G_{VIII} parent; one clone was unclassified. The 1:1 segregation ($0{\cdot}7 > P > 0{\cdot}5$) is what would be expected when a heterozygous stock (G_{12}, mt^{22}/mt^3) is crossed to a homozygous one (G_{VIII}, mt^y/mt^y) whose *mt* allele occupies a middle position in the dominance ladder of *mt* alleles: $mt^{22}\ldots > mt^y\ldots > mt^3\ldots$.

(ii) When the sensitive A^+G_{VI} stock was crossed to the mate-killer $A^- C_8$ stock, from 63 conjugating pairs only 63 exconjugant F_1 clones were isolated. This was because from the two co-conjugants of each pair only one exconjugant survived; the other invariably died shortly after it had separated from its mate, showing the typical signs of the mate-killing effect. This, together with the observations reported in Materials and Methods, indicates that (1) each pair represented an interstock mating and (2) all of the surviving clones were of cytoplasmic descent from the C_8 parent. As Table 1, section (*a*) shows, 42 out of 63 clones isolated survived and were examined. Of these clones 39 were typed and all were found to undergo autogamy. As before, the segregation of the mating-type marker gene revealed that cross-fertilization typically occurred. Twenty-three of the 42 clones examined expressed the mating type P-y of the G_{VI} parent, 6 expressed the mating type P-α of the C_8 parent, and 11 expressed a non-parental (nP) mating type, i.e. a mating type controlled by a *mt* allele carried as 'recessive' by one of the two parents; two clones were unclassified. Since C_8 and G_{VI} stocks turned out to have the mt^α/mt^β and mt^y/mt^1 genotype respectively, and the 'peck-order' dominance of the *mt* alleles was known to be $mt^y\ldots > mt^\alpha\ldots > mt^\beta\ldots > mt^1$, the observed segregation, which was not significantly different ($0{\cdot}5 > P > 0{\cdot}3$) from a 2:1:1 ratio for mating types P-y:P-α:nP, was as expected.

If we exclude the two exceptional A^- clones of $G_{VIII} \times G_{12}$, which most probably resulted from homotypic pairing (selfing) between A^- G_{12} cells, all of the F_1 clones from both original crosses were uniform with respect to their ability to undergo autogamy. The simplest interpretation of this result is that the F_1 offspring are the outcome of crosses between stocks one of which is dominant and the other homozygous recessive for genes at a particular locus or at several loci. However, a cytoplasmic control of autogamy cannot be ruled out. In fact, if the two members of each pair had regularly exchanged cytoplasm during conjugation, the uniformity observed among the F_1 clones would also be the expected outcome. The next cross-breeding experiments were made to discriminate between these two possibilities.

(*b*) *Backcrosses*

(i) An A^+ F_1 clone, designated 32-F_1, from the above described $G_{VIII} \times G_{12}$ cross was backcrossed to the A^- G_{12} parent (Table 1, section (*b*)). The 32-F_1 clone of mating type P-y, whose cytoplasm could be derived from either parent, was selected for further study because of its good mating reactivity and its high rate of autogamy (nearly 100% of the cells passed simultaneously through autogamy when fully mature cultures were fed an excess of food for 4–5 days and then starved). The B_1 viability was high and the observed segregation of 58 A^+ to 67 A^- clones was compatible ($0{\cdot}5 > P > 0{\cdot}3$) with the 1:1 ratio. As to the segregation of the mating-type marker gene, we knew that stock G_{VIII} of mating type P-y was homozygous for the mt^y allele and stock G_{12} of mating type P-22 carried the genotype mt^{22}/mt^3. Therefore, given the serial relation of dominance of the *mt* alleles reported in section (*a*) (i), the F_1 clones of mating type P-y from $G_{VIII} \times G_{12}$ should have had the genotype mt^y/mt^3. If this was true, one would expect the B_1 generations to express the mating types P-22, P-y and nP in a 2:1:1 ratio, respectively. This was observed with a good probability value ($P = 0{\cdot}5$), indicating that (1) the 32-F_1 clone was a true hybrid and (2) cross-fertilization should have occurred in the backcross.

(ii) The mate-killer trait segregated in the F_1 of the $G_{VI} \times C_8$ original cross produced both mate-killer and sensitive clones, as mating tests and labelling experiments clearly demonstrated (Luporini & Dini, 1975; Luporini & Dini, in preparation). An A^+ F_1 clone of mating type P-y, designated 9-F_1, was chosen from the sensitive progeny and backcrossed to the mate-killer C_8 parent (Table 1, section (*b*)). All of the 123 B_1 clones isolated belonged to the same cytoplasmic lineage of the A^- C_8 parent since they derived from exconjugants which had evidently expressed their mate-killing action against the sensitive conjugating partners. They fell into two phenotypic classes, autogamous and non-autogamous, as occurred in the backcross reported above in (i) but, at variance with this last, the backcross involving the 9-F_1 clones produced an excess of A^- progeny clones (26 A^+ to 45 A^-). The deviation from a 1:1 ratio was significant at the 5% level but not at the 1% level. The substantial B_1 death rate, as a result of which only 74 clones were examined of the 123 isolated, may have contributed

to the skewed ratio. The prediction with regard to the mating-type segregation consequent to a regular cross-fertilization was fulfilled. In fact, in no case did a mating type other than P-y or P-α appear in the B_1 progeny, and the numbers found agreed ($0{\cdot}7 > P > 0{\cdot}5$) with a 1:1 ratio. It will be recalled that the G_{VI} stock was of mating type P-y (mt^{γ}/mt^{1}), whereas the C_8 stock was of mating type P-α (mt^{α}/mt^{β}). Given the order of dominance of *mt* alleles reported in section (*a*) (ii), the $G_{VI} \times C_8$ progeny expressing the mating type P-y should, therefore, carry the genotype mt^{γ}/mt^{β} or mt^{γ}/mt^{α}. The latter genotype must be carried by the 9-F_1 clone involved in the backcross. This accounts for the B_1 segregation pattern observed and reinforces the cross-breeding origin of the 9-F_1 clone.

The major features of the inheritance of the autogamy trait emerging from the cross-breeding experiments reported in sections (*a*) and (*b*), respectively, are the following: (1) with only few exceptions all of the F_1 hybrid clones are A^+; (2) the segregation of A^+ and A^- clones in the B_1s approximates a 1:1 ratio. This is what one would expect in genetic inheritance of autogamy if the A^+ wild stocks G_{VIII} and G_{VI} were homozygous for a dominant allele (a^+/a^+), and the A^- wild stocks G_{12} and C_8 were homozygous for the recessive allele (a^-/a^-) at the single locus designed as the *a* (autogamy) locus.

(*c*) *Intercrosses*

The two-allele hypothesis postulated above predicts that an F_2 ratio of $3A^+$ to $1A^-$ clones must appear in the progenies of intercrosses between heterozygous (a^+/a^-) F_1 clones. Two intercrosses were set up between A^+F_1 progeny clones of $G_{VIII} \times G_{12}$ (Table 1, section (*c*)). Both had in common the clone 32-F_1 of mating type P-y which had shown a good mating reactivity when backcrossed to the A^- parental stock G_{12} (see section (*b*) i). Two clones, designated 27-F_1 and 28-F_1, respectively, that expressed the mating type P-22 were chosen as partners for 32-F_1. These clones were from one synclone, so that one clone was descended cytoplasmically from G_{VIII} and the other from the G_{12} parent. The intercrosses of 32-F_1 with 27-F_1 and 28-F_1 scored for autogamy gave quantitatively different results; the frequency of the A^+ clones was lower in the 32-$F_1 \times$ 28-F_1 intercrosses than in the other involving the sister clone 27-F_1. However, the Chi-square test for homogeneity of the two intercrosses indicated that the departure from homogeneity was statistically insignificant ($\chi^2 = 0{\cdot}21$, $P > 0{\cdot}5$), and the numbers found in each F_2 progeny fit the expectation of a 3:1 ratio for A^+: A^- clones. In both cases, control of the mating-type segregation showed F_2 clones to fall into two phenotypic classes, P-22 and P-y in approximately equal frequencies, in agreement with a 1:1 ratio. Since the 32-F_1 clone had the genotype mt^{γ}/mt^{3} (see section (*b*) i), these are the expected results attributing to 27-F_1 and 28-F_1 clones the same mt^{22}/mt^{γ} genetic constitution of true hybrids.

The weakness and/or the low reactivity of most of the F_2 clones hampered a further breeding analysis. In other words, it was not possible to determine whether the genetic constitution of F_2 individuals actually consisted of all of the

three possible combinations of a pair of alleles: a^+/a^+; a^+/a^-; a^-/a^-, in the ratio 1:2:1 as expected.

(B) *Genetic analysis of the exautogamous F_2 generations after crossing*

The hypothesis of a monogenic inheritance of the autogamy trait finds further support in the study of F_2 progenies obtained by autogamy. The A^+ 32-F_1 and 9-F_1 clones, respectively, derived from $G_{VIII} \times G_{12}$ and $G_{VI} \times C_8$ original crosses, were permitted to undergo autogamy. The two exautogamous F_2s obtained were examined at maturity for both the autogamy and mating-type segregations. The results are summarized in Table 2. Earlier, in section (A) (*a*, *b*), it was reported that both clones expressed mating type P-y, and the 9-F_1 clone was descended cytoplasmically from the A^- mate-killer C_8 parent, whereas the cytoplasmic origin of

Table 2. *Segregation of both autogamy and mating-type traits at F_2 by autogamy*

Original crosses	$G_{VIII} \times G_{12}$		$G_{VI} \times C_8$
Designation of F_1 clones passed through autogamy	32 – F_1		9 – F_1
Phenotype and genotype of the F_1 clones	P – y, mt^y/mt^3 A^+, a^+/a^-		P – y, mt^y/mt^α A^+, a^+/a^-
Viability at F_2 by autogamy (%)	67/124 (54)		56/121 (46)
Segregation of A^-:A^+ clones in exautogamous F_2 progenies*	6:55		4:47
Chi-square and probability†		$\chi^2 = 0{\cdot}13$ $0{\cdot}9 > P > 0{\cdot}7$	
Segregation of recessive: dominant mating type in exautogamous F_2 progenies*	5:57		2:48
Chi-square and probability†		$\chi^2 = 0{\cdot}78$ $0{\cdot}5 > P > 0{\cdot}3$	

* For the reasons previously reported in (A), in each progeny the total numbers of clones actually typed for either autogamy or mating-type trait differ between them and in respect to the total number of viable exautogamous F_2 clones.

† See Materials and Methods for the Chi-square test of homogeneity used.

the 32-F_1 clone was unknown. Their hybrid genetic make-up at the *mt* locus (32-F_1, mt^y/mt^3; 9-F_1, mt^y/mt^α) was ascertained by the mating-type segregation obtained from backcrosses. Likewise, their heterozygosity at the *a* locus (a^+/a^-) was inferred from the resulting data of the same backcrosses.

When the exautogamous F_2s from 32-F_1 and 9-F_1 were scored for autogamy, a small number of A^- clones, 6A^- *vs* 55A^+, and 4A^- *vs* 47A^+ was found to occur. From each exautogamous F_2 progeny, two A^- segregant clones showing mating type P-y were backcrossed to the respective A^- G_{12} (P-22) and C_8 (P-α) parental

stock. Despite the small number of clones checked (no more than 20) for each of the four B_2 progenies, no A^+ clone was observed to segregate. These results indicate that the A^- exautogamous F_2 clones were true segregants since they had apparently lost the ability to undergo autogamy; that is, according to our interpretation, their genotype at the *a* locus was a^-/a^-.

The results obtained with respect to the segregation of the mating type conformed to those of autogamy trait segregation. In fact, the F_2s from 32-F_1 and 9-F_1 clones showed 5 *vs* 57 and 2 *vs* 48, recessive *vs* dominant mating types, respectively. The segregations observed in the two exautogamous progenies for both autogamy and mating-type trait were subjected to a Chi-square test for homogeneity. In both cases the departure from homogeneity was statistically insignificant. Yet all the segregations significantly differed ($0{\cdot}001 > P$) from a 13:29 ratio for recessive:dominant phenotypes expected when a clone heterozygous at a single locus passes through autogamy (see Introduction); an excess of dominant phenotypes was constantly observed. This indicates that deficiencies (significant) of recombinations occur in the autogamy of *E. crassus*. However, the segregation ratios found here are comparable to those already obtained in similar cases by Luporini & Dini (1977) in the same species, as well as (although perhaps coincidentally) in the related species *Euplotes minuta* by Siegel & Heckmann (1966), Luporini & Nobili (1967), and Heckmann & Frankel (1968).

Regardless of the deviations noted, the important point to be emphasized here is the close similarity of the autogamy and mating-type segregation patterns. Under the same set of environmental conditions, similarity in segregation patterns, as a rule, indicates similarity in genetic control systems. Since it is well known that in *E. crassus* the mating-type trait is controlled by alleles at a single locus (Heckmann, 1964; see also Luporini & Dini, 1977), the inference that autogamy is also determined by one locus with a pair of alleles is justifiable.

(C) *Independence of the* mt *and* a *loci*

Except in the F_1 progenies from original crosses in which all clones were virtually A^+ regardless of the mating type expressed, in all later generations recombinations of parental traits occurred. The A^+ and A^- clones appeared to be distributed among the segregating mating-type classes with frequencies that suggested an independence of the genetic control system of these two cellular characteristics. This observation was substantiated by the statistical analysis of the results obtained at B_1 of 32-$F_1 \times G_{12}$ backcross. The cross was selected for analysis because of the substantial number of progeny clones obtained. As described above in (A), sections (*b* and *c*), the parents involved in the backcross showed the following phenotypes and genotypes at the *mt* and *a* loci: 32-F_1, mating type P-y (mt^y/mt^3), A^+ (a^+/a^-); G_{12}, mating-type P-22 (mt^{22}/mt^3), A^- (a^-/a^-). If the *mt* and *a* genes segregate independently, each of the three mating-type classes of the B_1 generation, P-22, P-y and nP, should consist of equal numbers of A^+ and A^- clones. Out of the 57 P-22 clones, 24 were A^+ and 33 A^-; out of the 33 P-y clones, 13 were A^+ and 20 A^-; out of the 34 nP clones, 20 were A^+ and 14 A^-. Linkage

was assessed by using a Chi-square test as described by Sinnot, Dunn &Dobzhansky (1950); the Chi-square value did not exceed 3·84 ($P = 0{\cdot}05$), proving that the *mt* and *a* loci are not closely linked. (The frequencies reported above were calculated based on a total of 124 clones, that is, only clones that could be typed for both autogamy and mating-type traits were included. For this reason, these frequencies do not correspond to those reported in Table 1, section (*b*)).

4. DISCUSSION

The results presented in this report support the conclusion that in *Euplotes crassus* the ability to undergo autogamy is genetically determined. Mendelian ratios observed in the offspring from cross-breeding experiments indicate a monogenic inheritance of the autogamy trait by a single pair of alleles which express a simple dominance relationship; the dominant allele (a^+) permits the expression of the potentiality for autogamy. The autogamous (A^+) stocks employed in the present research are homozygous for this allele (a^+/a^+), whereas the non-autogamous (A^-) stocks are homozygous for the recessive allele (a^-/a^-). The segregation pattern of the autogamy trait observed in the exautogamous F_2s from F_1 hybrid lines agrees with this model and provides the most rigorous demonstration of the nuclear localization of the autogamy-controlling gene. The analysis of combinations between autogamy and mating-type phenotypes in the cross-breeding progenies also agrees with this model, and the independence of the *a* (autogamy) and *mt* (mating type) loci is not surprising in view of the rather high number of chromosomes in the micronucleus of *Euplotes* (see Raikov, 1972). The appearance of two discrete phenotypic classes readily distinguishable in all offspring makes the possibility of a polygenic control of the autogamy trait in *E. crassus* unlikely. Yet the genetic data presented do not exclude the possibility of control by two or more closely linked genes. The findings of Siegel & Heckmann (1966) and Heckmann & Frankel (1968) appear to argue against these possibilities. They obtained circumstantial evidence of a single-gene basis for the autogamy trait in *Euplotes minuta*, a biological species closely related to *E. crassus* (Nobili *et al.*, 1978; Gates, 1978).

Finally, the deviation from the expected 1:1 ratio for A^+:A^- clones in the 9-F_1 $(G_{VI} \times C_8) \times C_8$ backcross (see Table 1, section (*b*)) deserves comment. Although other explanations for the skewed ratio may be considered, specific conditions of the cell environment causing deviation cannot be eliminated. This alternative hypothesis appears plausible if the pedigree of the B_1 progeny is considered. The B_1 progeny was, in fact, the final result of a mating protocol in which the sensitive A^+G_{VI} stock (a^+/a^+), as well as the sensitive A^+9-F_1 clone (a^+/a^-), were crossed to the mate-killer A^-C_8 stock (a^-/a^-) (see Results). Hence, both the F_1 and B_1 generations had their cytoplasm descended from the A^-C_8 parent, and the dominant a^+ allele was introduced into the B_1 clones by two transfers into an increasingly non-autogamous genetic background of stock C_8. Since the deviation observed was due to an excess of A^-B_1 clones, it may be inferred that the a^+ allele removed

from the genome of origin and transferred into a non-autogamous, cytoplasmic and genetic background largely of stock C_8, is occasionally not expressed. There are precedents in various organisms for the unstable behaviour of genes (see Nanney, 1958). In particular, the studies of Smith & Sand (1957) and Sand (1957) on interspecific hybrids of *Nicotiana* suggest that genes removed from the genome of origin may be incapable of responding regularly to normal developmental stimuli in a foreign genome. In this connexion it should be mentioned that the A^+ and A^- stocks of *E. crassus* displayed signs of incompatibility indicating an incipient evolutionary divergence (Luporini & Dini, 1977). Since the low mating reactivity and low survival frequency after sexual reorganization precluded using the progeny clones in further breeding experiments, the actual causes of the deviation must be established by other means.

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